In this study, we analyze the structures of smaller molecules bound to ribosome, such as tRNA and elongation factors, which are included in the EM density maps of the 70S ribosome, by building atomic models of these molecules in the bound state. The structures of these bound molecules may be different from those in the isolated state or the X-ray crystal structures. In this analysis, we need to spot these molecules in the EM density map, which is occupied mostly by the 70S ribosome. By fitting the atomic model of the 70S ribosome into the EM density map and eliminating the density of the regions overlapped with the atomic model, we are able to extract the regions for the bound molecules. Our results show that the best-fitting atomic model of the 70S ribosome built in our previous sturdy can extract the regions for the bound molecules more clearly than the original PDB structure.

1366-Pos
Spontaneous Vs. Allosteric Dissociation of E-Site tRNA During Polypeptide Elongation
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During ribosome-catalyzed polypeptide chain elongation, dissociation of the deacylated tRNA from the E-site has been proposed to be either spontaneous or triggered allosterically by binding of the next cognate ternary complex to the A-site. Using fluorescent labeled tRNAs, we have measured single molecule fluorescence intensities and single molecule FRET between adjacent tRNAs in the ribosome. From these measurements we have been able to determine tRNA occupancy in the ribosome, and thus whether E-site tRNA dissociates before or after A-site occupancy. In the former case there are a maximum of 2 simultaneously bound tRNAs, while the latter case results in transient binding of 3 tRNAs simultaneously. In a total internal reflection florescence microscope, ribosomes were attached to glass microscopes slides via a biotinylated mRNA coding for MRFFRFY.... (single letter amino acid sequence). When synthesis was initiated with $tRNA^{fMet}$ fully charged with formylated-Met, 60-70% of the ribosomes lost their E-site tRNA prior to ternary complex binding (2-tRNA pathway) at the 2nd and 3rd elongation cycles (R and F respectively). In contrast, for synthesis initiated with uncharged initiator $tRNA^{fMet}$, >90% of the ribosomes followed the 2-tRNA pathway in the 2nd cycle, but only ~15% in the 3rd cycle (~75% following the 3-tRNA route, 10% not categorized). In cycles 4 and 5, almost all ribosomes followed the 2-tRNA pathway. Thus, the length of the peptide chain and/or the specific amino acids bound to the P-site tRNA strongly influence the allostery of E-site tRNA dissociation. Such allostery is also sensitive to the presence or absence of polyamines, Mg^{2+} concentration, and the specific codons in positions 2 and 3. Supported by NIH R01grant GM080376 and NIST ATP grant 70NANB7H7011 through Anima Cell Metrology, Inc.

1367-Pos
Enhancement of Single Molecule Fluorescence Signals by Colloidal Silver Nanoparticles in Studies of Ribosome Dynamics
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Metal enhanced fluorescence (MEF), in which a surface plasmon near a noble metal alters the spectral properties of an organic fluorophore, increases fluorescence intensity without a concomitant increase in photobleaching rate. To improve recordings of single molecule fluorescence signals from individual ribosomes, we studied the emission of Cy3- and Cy5-labeled ribosomes and tRNAs attached near 50-80 nm silver colloidal particles on a glass microscope coverslip. The fluorescence of Cy3 and Cy5 labeled initiation complexes (ICs) near 50 nm silver particles was increased 4- and 5-fold, respectively, compared to labeled ICs in the absence of silver colloids. Photobleaching lifetime was not significantly accelerated, resulting in 4-5 fold enhancement of total photon emission before photobleaching. Fluorophores showing enhanced fluorescence were colocalized with the colloidal particles, as detected by light scattering. Other ribosomes or tRNAs that were farther away had intensities similar to those on plain glass. Aggregates of silver colloidal particles themselves produced wavelength-shifted luminescence similar to fluorescence, presumably due to resonance between nearby metal particles. With ribosomes bound to the glass substrate near the silver particles via a short mRNA, interaction between $tRNA^{Arg}$-Cy3 in the ribosomal P-site and fMet-Arg-Phe-$tRNA^{Phe}$-Cy5 in the A-site had FRET efficiency and dynamics similar to ribosomes farther away and on plain glass. Binding of Cy5-Arg-$tRNA^{Arg}$ to ICs labeled with Cy3 on the large subunit protein L11, in the absence of the translocase EF-G, produced FRET efficiency and dynamics characteristic of specific codon-dependent A-site binding. Addition of EF-G reduced FRET efficiency, as expected. These tests demonstrate that the colloidal silver nanoparticles increase fluorescence and total photon emission without compromising the biophysical activity of ribosomes.
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1368-Pos
Establishing a Fluorescence-Based Helicase Assay for Monitoring Eukaryotic Protein Synthesis Initiation
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Efficient mRNA recruitment to the human ribosome requires a region of single stranded RNA close to the cap structure. The eukaryotic initiation factor 4A (eIF4A) is a DEAD box helicase that is essential for unwinding any stable secondary structure that would inhibit this step. Although studied for many years, the majority of helicase assays involving eIF4A and its associated proteins have failed to rigorously analyze the kinetic events that occur during mRNA unwinding. To provide this kinetic understanding we have developed a continuous fluorescence-based assay to measure RNA duplex unwinding events. This assay utilizes an RNA oligonucleotide modified with cyanine 3 (Cy3) annealed to a complimentary strand modified with black hole quencher (BHQ). Separation of the RNA duplex region significantly enhances the Cy3 fluorescence, enabling us to measure RNA helicase activity in real time by fluorescence spectroscopy. Data will be presented to show how we are using this assay to determine the kinetic role of accessory factors on the helicase activity by eIF4A. Moreover, we show how this data combined with a continuous coupled ATPase assay is enabling us to determine the relationship of ATP hydrolysis to the unwinding of duplex RNA by this DEAD box helicase.
We will also present data employing RNA duplexes of different lengths, allowing us to understand how the processivity of eIF4A is influenced by other initiation components.
This study will provide us with the foundation to begin understanding the kinetic framework of mRNA recruitment to the human ribosome.

DNA, RNA Structure & Conformation I

1369-Pos
Structure and Mechanism of the glmS Ribozyme
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The self-cleaving glmS ribozyme is a mechanistically unique functional RNA among both riboswitches and RNA catalysts as its catalytic activity provides the basis of genetic regulation and depends upon glucosamine-6-phosphate (GlcN6P) as a coenzyme. A substantial body of biochemical and biophysical data relating the structure and function of the glmS ribozyme has been amassed, in our laboratory and others, in a relatively short period of time since its discovery. However, a precise and comprehensive mechanistic understanding of coenzyme function in glmS ribozyme self-cleavage has not been elaborated. Careful consideration of the available biochemical and biophysical data relating the structure and function of the glmS ribozyme necessitates that general acid and general base catalysis in a coenzyme-dependent active site mechanism of RNA cleavage are inherently interdependent. We propose a comprehensive mechanistic model wherein the coenzyme, GlcN6P, functions both as the initial general base catalyst and consequent general acid catalyst within a proton-relay thus fulfilling the apparent biochemical requirements for activity. This analysis in combination with other considerations regarding the effects of coenzyme binding on riboswitch structure and function suggests that the development of glmS ribozyme agonists as prospective antibiotic compounds must satisfy strict chemical requirements for binding and activity.

1370-Pos
Antibiotic Development by Investigation of the glmS Riboswitch
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Although bacterial infections have always been of significant interest to researchers and physicians, the drug-resistant bacterial strains that have recently developed are causing new concerns and are much more difficult to combat. Our current methods for treating bacterial infections include broad-spectrum

antibiotics which target only a small number of bacterial processes. However, with the discovery of riboswitches, we are developing new ways to fight bacterial infections which make use of their own natural metabolic pathways, essentially causing bacteria to destroy themselves. Riboswitches are found in non-coding regions of messenger RNAs and these RNA elements bind to ligands to control the expression of nearby genes. The glucosamine-6-phosphate (glmS) riboswitch is unique in that upon binding its ligand, glucosamine-6-phosphate (GlcN6P), it undergoes self-cleavage and is therefore also a catalytic RNA. The cleavage event targets the RNA for subsequent degradation, thereby repressing further gene expression. To study the glmS riboswitch, initial experiments were performed to determine the mechanism followed upon binding of the natural ligand. Since then, analogs of the natural ligand have been obtained and are being tested for their catalytic capabilities through kinetic analyses and rate constant calculations. Once successful candidates have been determined, these non-natural ligands will be introduced into live bacterial cultures, hopefully disrupting normal cell metabolism and reproduction. If successful, these analogs could be used as novel antibiotics, offering a more specific mode of targeting a wide variety of bacterial species.

1371-Pos

Folding of the Thiamine Pyrophosphate (TPP) Riboswitch

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TPP riboswitches regulate the expression of thiamine-synthesis (*thi*) genes through a variety of mechanisms, all of which involve binding of TPP to a structured aptamer formed in the untranslated region (UTR) of a *thi* mRNA. We used a high-resolution, single-molecule optical trapping assay to characterize mechanically the folding of the TPP riboswitch aptamer located in the 3'UTR of the *thiC* gene of *Arabidopsis thaliana*. Each RNA molecule, containing either the complete aptamer sequence or a portion thereof, was transcribed *in vitro,* annealed to DNA handles via single-stranded overhangs, and placed in a ''dumbbell'' experimental geometry[1]. By applying tension to the ends of the RNA molecule under equilibrium conditions and measuring the corresponding extensions, we observed transitions among several well-defined folding states, which we discuss in the context of secondary and tertiary structures formed by the aptamer[2]. One low-force state of the full aptamer, corresponding to the formation of structural elements located near the three-helix junction, was abolished by mutating a single nucleotide believed to participate in specific tertiary contacts within the junction[2,3]. We observed that the mutant aptamer does not bind TPP or other substrates (thiamine, thiamine monophosphate), and that the wild-type aptamer only binds substrates concomitant with entry into the fully-folded state. We also studied the energetics of substrate binding under non-equilibrium conditions by rapidly increasing or decreasing the extension of the aptamer and measuring the hysteresis in force. The number of phosphates on the substrate modulated the amount of work required to induce substrate unbinding, the height and location of the energy barrier to substrate unbinding, and the amount of RNA released.

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1372-Pos

Structure and Function of a Potential Mammalian Riboswitch

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Riboswitches, found in untranslated regions of mRNAs, bind to specific cellular metabolites and undergo a conformational change which modifies expression of a nearby coding region of the mRNA. This coding region is involved in the synthesis of the same metabolite, thereby providing an efficient feedback mechanism of genetic control. To date, various riboswitches have been described to effectively control genetic expression in bacterial cells, but none have been discovered in mammals. We are investigating the structure and function of a potential mammalian riboswitch, thought to control polyamine biosynthesis. Polyamines surround cellular DNA to stabilize the DNA negative charge. To validate this small RNA as a new riboswitch, we are using in-line probing to verify specific metabolite binding and subsequent conformational change. Additionally, to verify the ability of the potential riboswitch to control gene expression, in vivo studies are being performed using a reporter gene system. Successful results from both of these investigations will determine whether this small RNA is a true riboswitch. Further investigations will include determination of its tertiary structure. It is known that cancer cells require a higher concentration of polyamine due to their increased replication rate. Thus, a combination of structural and functional studies of this RNA may prove useful in the development of novel cancer therapies.

1373-Pos

Dynamics of the Catalytic Pocket of a Diels-Alder Ribozyme

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The Diels-Alder ribozyme, an in vitro-evolved highly abundant ribonucleic acid enzyme, accelerates the formation of carbon-carbon bonds between a diene and a dienophile in a [4+2] cycloaddition reaction, a reaction with broad application in biochemistry and organic chemistry.

We have examined the ribozyme in the unbound form in solid and liquid phase by means of Molecular Dynamics simulations of 1 microsecond total simulation time. Our simulations confirm highly dynamic state of the catalytic pocket as observed by recent NMR spectroscopy studies.

However, the preformed catalytic pocket architecture, suggested previously based on X-ray investigations, exists only under certain conditions. Simulations of the crystal state show that at the temperature of 100K the catalytic pocket remains in its starting conformation. Yet, at the transitional temperature of 250K a collapse of the catalytic pocket occurs, and the ribozyme adopts an ezymatically inactive closed conformation of the pocket.

Simulations in solution performed at 300K at different magnesium ions concentration reveal that the stabilization of the catalytic pocket depends on high amounts of Mg-ions. At higher Mg^{2+} concentrations the cations are more likely to bind to the backbone of those residues that bridge the opposite strands of the pocket, which leads to stabilization of the enzymatically active open conformation. Simulations with artificial constraints confirm and quantify the effect of backbone stabilization on a catalytically active state. At too low Mg-ion concentrations, catalytically inactive states with a collapsed catalytic pocket dominate. In these conformations the ribozyme is not able to host any reactant. The catalytically active state with an open pocket is a metastable state that can only be accessed and is only sufficiently stabilized at a high enough magnesium concentration, explaining the experimentally found full catalytic activity dependence on the Mg-ions concentration.

1374-Pos

Structural Probing of the T Box Antiterminator-tRNA Complex

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Structural changes in a unique RNA-RNA binding interaction were probed using 2-aminopurine. The 5′-untranslated leader region (5′-UTR) of the T box family of genes folds into a structure that selectively recognizes a specific tRNA through two unique base-pairing events. The first involves base pairing between the anticodon of cognate tRNA and a tri-nucleotide sequence (specifier sequence) in the specifier loop of stem 1 in the 5′-UTR. The second base pairing event involves the non-aminoacylated tRNA acceptor end base pairing with the first four nucleotides at the 5′-end of a bulge in a highly conserved antiterminator element. In the absence of the stabilization of the uncharged tRNA acceptor end base pairing to the antiterminator element, the more thermodynamically stable terminator element forms and transcription terminates. In this manner, the leader region specifically recognizes cognate tRNA and responds structurally to the charging ratio of the tRNA to regulate transcription, thus making the T box mechanism an example of a riboswitch. Interestingly, the predicted thermodynamic stabilization provided by the four base pairs between the tRNA acceptor end and the antiterminator is not sufficient to overcome the predicted stability difference between the antiterminator and the terminator elements. Consequently, additional structural factors likely play a role in stabilizing the resulting complex. The structural changes induced in both the antiterminator element and the tRNA were investigated using a model system to determine what additional factors, beyond base pairing, contribute to stabilization of the resulting tRNA-antiterminator complex. Fluorescence monitoring of the base analog 2-aminopurine at select positions throughout a model complex indicated that binding results in an induced-fit and a highly stacked environment at the binding interface. These structural features contribute to the overall stabilization of the complex beyond the four base pairs.

1375-Pos

Nanosecond Motions of the Substrate-Recognition Duplex in a Group I Intron Assessed by Site-Directed Spin Labeling

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The *Tetrahymena* group I intron recognizes its oligonucleotide substrate in a two-step process. First, a substrate recognition duplex, called the P1 duplex,